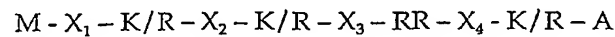26

CLAIMS

1.   A recombinant polynucleotide, the polynucleotide comprising a first and a
second sequence, the first sequence encoding a signal peptide comprising a TAT signal
and a Sec avoidance signal and the second sequence encoding a heterologous protein,
5   wherein the sequence of the signal peptide is

$$M - X_1 - K/R - X_2 - K/R - X_3 - RR - X_4 - K/R - A$$

in which X_1 is a sequence of 0 to 10 amino acids;

X_2 is a sequence of 0 to 3 amino acids;

X_3 is a sequence of 0 to 10 amino acids; and

10   X_4 is a sequence of 15 to 24 amino acids in which at least 75% up to about
90% of the residues are hydrophobic.

2.   A recombinant polynucleotide according to claim 1 wherein X_1 is a sequence
of 0 to 5 amino acids, and is preferably 0.

3.   A recombinant polynucleotide according to claim 1 or 2 wherein X_2 is a
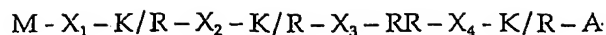15   sequence of 0 or 1 amino acid, preferably 0.

4.   A recombinant polynucleotide according to any one of claims 1 to 3 wherein
X_3 is a sequence of 0 to 5 amino acids, preferably 0.

5.   A recombinant polynucleotide according to any one of claims 1 to 4 wherein
X_4 is a sequence of at least 20 amino acids of which at least 18 are hydrophobic amino
20   acids.

6.   A recombinant polynucleotide according to any one of claims 1 to 5 wherein
X_4 is 23 amino acids.

7.   A recombinant polynucleotide according to any one of claims 1 to 6 wherein
the sequence of the signal peptide is MKKRRVVNSVLLLLLLASALALTVAPMAKA
25   (SEQ ID NO: 1).

8.   A signal peptide, the signal peptide having the sequence

$$M - X_1 - K/R - X_2 - K/R - X_3 - RR - X_4 - K/R - A$$

in which X_1 is a sequence of 0 to 10 amino acids;

X_2 is a sequence of 0 to 3 amino acids;

X_3 is a sequence of 0 to 10 amino acids; and

X_4 is a sequence of 15 to 24 amino acids in which at least 75% up to about 90% of the residues are hydrophobic.

9.   A signal peptide according to claim 8 wherein X_1 is a sequence of 0 to 5 amino acids, and is preferably 0.

10.   A signal peptide according to claim 8 or 9 wherein X_2 is a sequence of 0 or 1 amino acid, preferably 0.

11.   A signal peptide according to any one of claims 8 to 10 wherein X_3 is a sequence of 0 to 5 amino acids, preferably 0.

12.   A signal peptide according to any one of claims 8 to 11 wherein X_4 is a sequence of at least 20 amino acids of which at least 18 are hydrophobic amino acids.

13.   A signal peptide according to any one of claims 8 to 12 wherein X_4 is 23 amino acids.

14.   A signal peptide according to any one of claims 8 to 13 wherein the sequence of the signal peptide is MKKRRVVNSVLLLLLLASALALTVAPMAKA (SEQ ID NO 1).

15.   A method of producing a heterologous polypeptide from a host cell comprising a TAT translocation system, the method comprising:

(i)   transforming the host cell with a DNA sequence encoding the heterologous polypeptide and a signal peptide wherein the signal peptide comprises a TAT signal and a Sec avoidance signal wherein the sequence of the signal peptide is
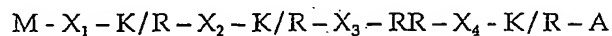
$$M - X_1 - K/R - X_2 - K/R - X_3 - RR - X_4 - K/R - A$$

in which X_1 is a sequence of 0 to 10 amino acids;

28

X_2 is a sequence of 0 to 3 amino acids;

X_3 is a sequence of 0 to 10 amino acids; and

X_4 is a sequence of 15 to 24 amino acids in which at least 75% up to about 90% of the residues are hydrophobic.

5   (ii)   culturing the host cell under conditions which allow expression of the heterologous polypeptide; and

       (iii)   recovering the heterologous polypeptide secreted from the host cell via the TAT translocation system.

16.   A method according to claim 15 wherein X_1 is a sequence of 0 to 5 amino
10   acids, and is preferably 0.

17.   A method according to claim 15 or 16 wherein X_2 is a sequence of 0 or 1 amino acid, preferably 0.

18.   A method according to any one of claims 15 to 17 wherein X_3 is a sequence of 0 to 5 amino acids, preferably 0.

15   19.   A method according to any one of claims 15 to 18 wherein X_4 is a sequence of at least 20 amino acids of which at least 18 are hydrophobic amino acids.

20.   A method according to any one of claims 15 to 19 wherein X_4 is 23 amino acids.

21.   A method according to any one of claims 15 to 20 wherein the sequence of the
20   signal peptide is MKKRRVVNSVLLLLLLASALALTVAPMAKA (SEQ ID NO: 1).

22.   A method according to any one of claims 15 to 21 wherein the host cell is Bacillus sp.

23.   A method according to claim 22 wherein the host cell is selected from the group consisting of Bacillus choshinensis, Bacillus brevis, Bacillus subtilis, Bacillus
25   licheniformis, and Bacillus megatorium.

24.   A method according to claim 22 wherein the host cell is Bacillus choshinensis.

25.   A method according to any one of claims 15 to 24 wherein the heterologous polypeptide is a polypeptide which readily folds in the cytoplasm.

26.   A method according to any one of claims 15 to 25 wherein the polynucleotide encoding the mature polypeptide has a sequence selected from:

5   (i) a sequence of nucleotides shown in SEQ ID NO:29 from nucleotide 85 to 1155;

(ii) a sequence that hybridises to SEQ ID NO:29 from nucleotide 85 to 1155 under conditions of high stringency;

(iii) a sequence which is greater than 90% identical to SEQ ID NO:29 from 10   nucleotide 85 to 1155; and

(iv) a sequence that encodes the amino acid sequence provided in SEQ ID NO:30 from residue 29 to 384.

27.   A method according to any one of claims 15 to 25 wherein the mature heterologous polypeptide comprises the sequence provided in SEQ ID NO:30 from 15   residue 29 to 384; or a polypeptide which is greater than 90% identical to the sequence provided in SEQ ID NO:30.

28.   A substantially purified polypeptide produced according to the method of any one of claims 15 to 27.

29.   A vector comprising the recombinant polynucleotide according to any of 20   claims 1 to 8.

30.   A host cell comprising the recombinant polynucleotide according to any of claims 1 to 8.